# Assessment of the genotoxicity of a titanium dioxide nanomaterial using a combination of *in vitro* and *in vivo* assays

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## BACKGROUND

Human exposure to manufactured nanomaterials such as titanium dioxide ($TiO_2$), often used in sunscreens and cosmetics, has increased worldwide. Their specific properties, such as size and high surface area/mass, render them attractive for many applications, but may also be associated to higher toxicity in biological systems and adverse health effects.

## OBJECTIVES

**To analyse the potential genotoxic effects of a well-characterized $TiO_2$ nanomaterial, correlating in vitro and in vivo effects.**

## MATERIALS & METHODS

**Dispersion Of $TiO_2$ by Sonication**

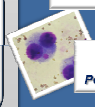
- Developed within the Nanogenotox Joint Action (Jensen et al., 2011) used for the NMDs preparation, in order to avoid aggregation/agglomeration of the nanoparticles.
- Detailed physicochemical characterization of the tested NMDs and exposure media was performed by electron microscopy, X-ray diffraction, BET, DXA, Raman spectroscopy, thermogravimetry, EDS and DLS (Nanogenotox unpublished results).

**Statistical Analysis**

- **Cytokinesis-block micronucleus assay** results from 2000 binucleated cells were analysed using the 2-tailed Fisher's exact test;
- **Cytokinesis block proliferation Index (Mean CBPI)** was performed by one-way ANOVA or Kruskal-Wallis test;
- **Mammalian Erythrocyte Micronucleus** results were analysed using the Chi-square test;
- **Percentage of DNA in Tail (%DNA in tail)** of BEAS-2B cells was analyzed by one-way ANOVA (with post Hoc Tukey's HSD test) and by Kruskal-wallis test on liver and spleen of mice;
- **Regression analysis** was used to try to adjust the dose-response curves to a mathematical function.

### *In vitro* Assays

**Cytokinesis-block micronucleus assay** (OECD 487 guideline) | **Comet Assay**



Primary Cultures of Human Peripheral Blood Lymphocytes | Human Bronchial Epithelial Cells BEAS-2B

### *In vivo* Assays

**Exposure of groups of mice to $TiO_2$**

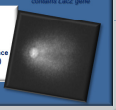
Exposure to 0,10 or 15 mg/kg, 2x

42h after last i.v. | 28 days after last i.v.

C57Bl6J mice harboring pUR288 plasmid that contains LacZ gene

**Mammalian Erythrocyte Micronucleus Test in tail blood** (OECD 474 guideline)

**Comet assay in liver and spleen** (Analysis of DNA damage using fluorescence microscopy and Comet Imager Software)



## RESULTS

### *In vitro* Assays

#### Comet Assay

- Significant induction of DNA damage (3-fold increase) at doses 32, 64 and 128 µg/cm² for %DNA in tail ($p$= 0.005, 0.002 and 0.002, respectively)
- No dose-dependent relationship

#### Cytokinesis-block micronucleus assay

- No chromosome breaks or loss were induced by $TiO_2$
- No impairment of cells proliferation ability, as measured by CBPI
- Significant increase in micronucleated binucleated cells (MNBC) frequency at concentration of 125 µg/ml ($p$= 0.038)
- Cell cycle progression (measured by CBPI) showed no significant decrease following exposure of lymphocytes

### *In vivo* Assays

#### Comet Assay

No significant induction of DNA damage in liver and spleen, measured by the % of DNA in tail, was observed after 28 days exposure to $TiO_2$

#### Mammalian Erythrocyte Micronucleus test

No significant induction of micronuclei was observed neither in mature (ERY) nor immature (RET) blood erythrocytes after 42h exposure to $TiO_2$

## CONCLUSIONS

- Different results were obtained *in vitro* depending on the assay: DNA damage was observed in BEAS-2B cells, but no significant clastogenic/aneugenic events were observed neither for BEAS-2B nor for human primary lymphocytes.
- *In vivo assays* (two endpoints in the same animal) revealed negative results for both endpoints tested.
- The data obtained for micronuclei analysis suggest a good comparability between the *in vitro* and *in vivo* outcomes, pointing to the absence of genotoxic effects following exposure to this $TiO_2$ nanomaterial at the dose levels tested.
- However, somewhat different genotoxicity outcomes concerning comet assay *in vitro and in vivo* may reflect tissue-specific effects affecting, e.g., cellular uptake of the nanomaterial.

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